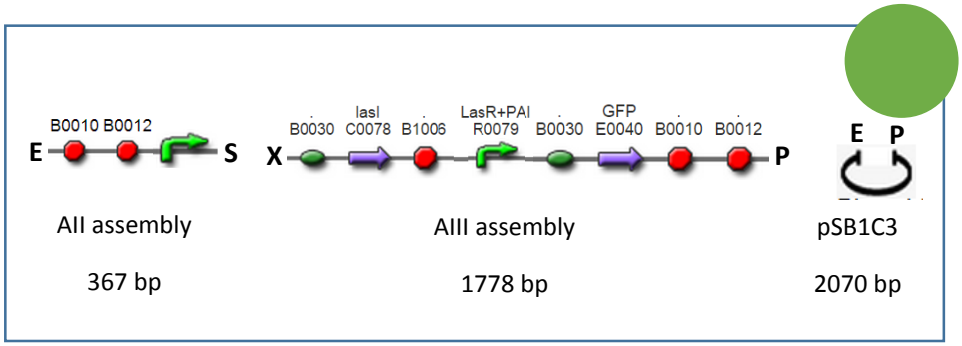
Assembly:

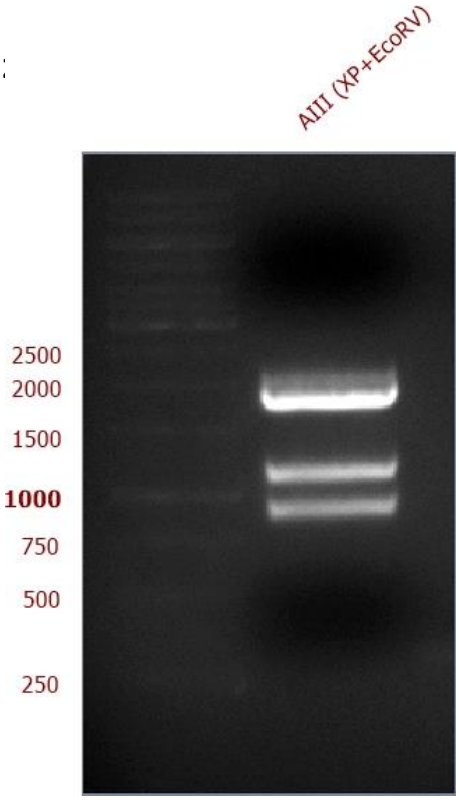
B II



1st Day

EXSP Digestion (see **Enzymatic Digestion Protocol**)

Parts	ng/ul	Volume to 2,5 ug (ul)	Buffer x10 (ul)	EcoRV (ul)	EcoRI (ul)	XbaI (ul)	SpeI (ul)	PstI (ul)	H_2O to 50ul (ul)	
AII assembly	~200	**~6 ug** = 30 ul	10		2	-	2	-	56	Split into 2 reactions of 50 ul
AIII assembly	~200	**~3,5 ug** = 17 ul	5	1	-	1	-	1	24	
pSB1C3	107,3	24,3	5		1	-	-	1	20,7	Repeat this digestion only if you run out of stock



EcoRV digests only the pSB1C3 plasmid generating 2 smaller fragments with 920 and 1150 bp. This procedure make it easier to purify assemblies with the size close to the 2070 bp of the pSB1C3.

Gel Purification

- See **Kit Wizard SV gel and PCR clean up Promega Protocol**
- Quantify digestion products

Parts	ng/ul	260/280
AII assembly (ES)	10	1,88
AIII assembly (XP)	18,6	1,95
pSB1C3 (EP)	24,3	2,83

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation (see **Ligation Protocol**)

Linear Plasmid 50 ng	2 ul	
Insert : Plasmid 3:1 (AII) ; 3:1 (AIII)	AII	AIII
	6 ul	8 ul
10x T4 DNA Buffer	2 ul	
T4 DNA ligase 1-5 u	1 ul	
H_20 to 20 ul	1 ul	

Obs: To determinate the amount of DNA necessary we used the following equation

$$Insert\ ng = plasmid\ ng \times \frac{insert\ bp}{plasmid\ bp} \times insert{:}plasmid\ ratio$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

3rd Day

Transformation (see **Transformation Protocol in *Escherichia coli* DH5-α**)

Organism: *E. coli* DH5-α

Selection: Cloranphenicol

4th Day

- Inoculate 3 – 4 colonies in a 6 ml LB with the same antibiotic used in the transformation protocol.
- Incubate overnight at 275rpm/37°C.

5th Day

Miniprep

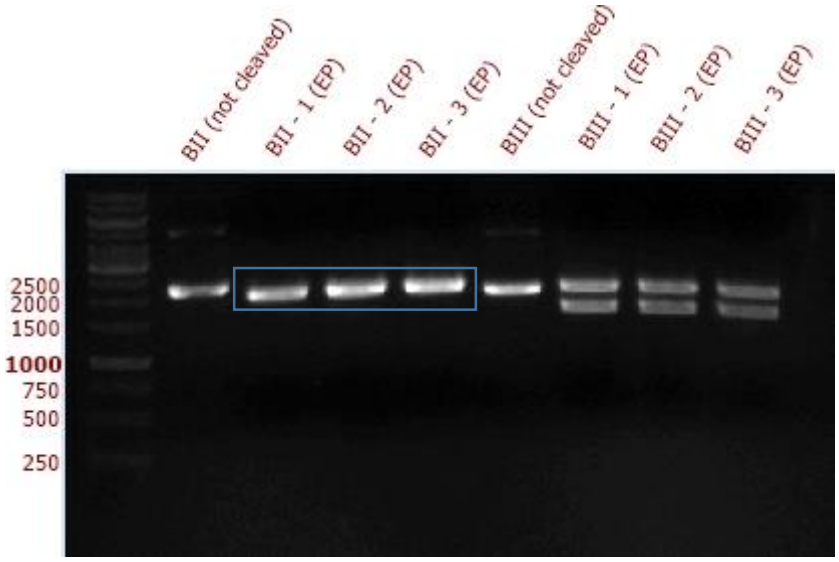
- Prepare **glycerol stock** of the clones (500ul glycerol 40% + 500ul inoculum).
- Extract plasmidial DNA (see **Alkaline Lyses or PureLink Invitrogen Protocol**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

Assembly Confirmation

- EP Digestion (see **Enzymatic Digestion Protocol**)

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	EcoRI (ul)	PstI (ul)	H_2O to 10ul (ul)
BII – 1	2	1	0,5	0,5	5
BII – 2	2	1	0,5	0,5	5
BII – 3	2	1	0,5	0,5	5

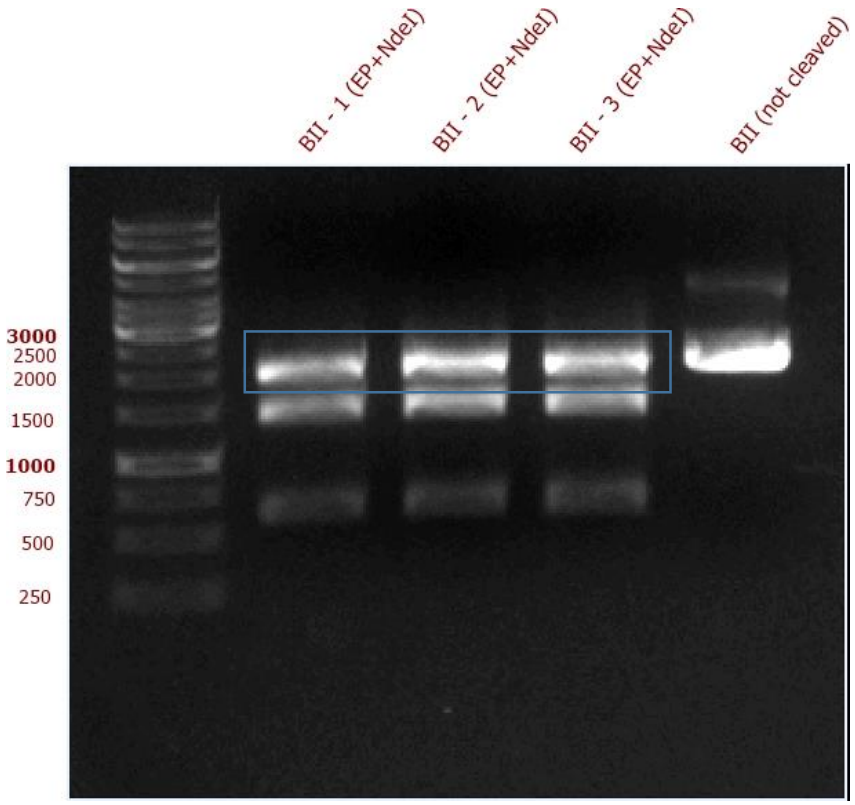
- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the EP digestion



The 2145 (BII assembly) and the 2070 bp (pSB1C3) frangments were to close in length to be properly separated in the electrophoresis gel. To deal with this problem we performed a triple digestion using EcoRI, PstI and NdeI. This last enzyme cuts only the BII assembly generating two different fragments, one with 625 bp and another with 1570 bp.

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	XbaI (ul)	NdeI (ul)	PstI (ul)	H_2O to 10ul (ul)
BII – 1	2	1	0,5	0,5	0,5	5,5
BII – 2	2	1	0,5	0,5	0,5	5,5

BII – 3	2	1	0,5	0,5	0,5	5,5



Size expected	Size in gel
2145 bp	~ 2200 bp